

-28-

CLAIMS

1. A lentivirus-based retroviral vector wherein the vector comprises: at least a portion of a lentivirus genome; a disrupted *gag* and a disrupted *pol* gene such that the vector is rendered replication-defective; a disrupted *env* gene; and a mutational cassette, wherein the mutational cassette comprises sequences encoding: a mutation target promoter sequence; a genomic source comprising a mutation target gene wherein there is a number of base pairs in the mutation target gene; an internal ribosome entry site for expression of a selectable marker gene; and a selectable marker gene.
2. The vector recited in claim 1 wherein the vector is transduction- and infection-competent.
3. The vector recited in claim 1 wherein the lentivirus genome is selected from the group consisting of human immunodeficiency virus type 1, human immunodeficiency virus type 2, feline immunodeficiency virus, simian immunodeficiency virus, visna-maedi virus, caprine arthritis-encephalitis virus, equine infectious anemia virus, bovine immune deficiency virus, and hybrids thereof.
4. The vector recited in claim 1 wherein the lentivirus genome comprises human immunodeficiency virus type 1.
5. The vector recited in claim 1 wherein the selectable marker comprises a positive selectable marker.
6. The vector recited in claim 1 wherein the selectable marker comprises a hygromycin B resistance gene.
7. The vector recited in claim 1 wherein the selectable marker comprises a hygromycin B phosphotransferase gene.

-29-

8. The vector recited in claim 1 wherein the mutation target gene comprises greater than 300 base pairs.

9. The vector recited in claim 1 wherein the mutation target gene comprises greater than 500 base pairs.

10. The vector recited in claim 1 wherein the mutation target gene comprises greater than 700 base pairs.

11. The vector recited in claim 1 wherein the mutation target promoter gene comprises a human cytomegalovirus promoter, and the mutation target comprises a thymidine kinase gene.

12. The vector recited in claim 1 wherein the genomic source comprising a mutation target gene comprises a human herpes virus type 1 gene.

13. A cell comprising the lentivirus-based retroviral vector as recited in claim 1.

14. The cell recited in claim 13 wherein the cell is a dividing or non-dividing eukaryotic cell.

15. The cell recited in claim 14 wherein the cell is a dividing cell.

16. The cell recited in claim 14 wherein the cell is a non-dividing cell.

17. An assay for determining a mutation frequency and a mutation rate of a retrovirus comprising:

- a) constructing the vector recited in claim 1;
- b) stably transfecting cells from a cell culture with the vector from (a)
- c) placing the cells under selection with a medium selectable for the selectable marker to produce a quantity of cell clones which contain an integrated vector;

-30-

- d) transiently transfecting the quantity of cell clones with a set of helper plasmids to produce a vector virus, wherein the set of helper plasmids contain a complement of structural genes which permit replication;
- e) infecting naïve cells from a cell culture with the vector virus;
- f) placing the cells from (e) under selection with the medium selectable for the selectable marker;
- g) cloning the cells from (f) to produce a quantity of initiator cell clones, wherein each of the quantity of cell clones is designated as an Initiator Clone (IC);
- h) confirming that the mutation target gene is functional in each IC and sequencing the mutation target gene for later comparison to mutation target genes which have undergone a cycle of replication;
- i) transiently transfecting the Initiator Clones with a set of helper plasmids to produce a vector virus;
- j) infecting naïve cells from a target cell culture with the vector virus from (i) to produce a quantity of infected target cells;
- k) placing a first portion of the quantity of infected target cells under selection with the medium selectable for the selectable marker and a substantially similar second portion of the quantity of infected target cells under selection with the medium selectable for the selectable marker plus a selective medium for the mutation target gene; wherein a number of drug resistant colonies grows from the first and second portions, and
- l) determining a viral titer for the first portion and a viral titer for the second portion by counting the number of drug-resistant colonies;

-31-

wherein the mutation frequency is calculated by dividing the viral titer of the second portion by the viral titer of the first portion, and the mutation rate is calculated by dividing the mutation frequency by the number of base pairs in the mutation target gene.

18. The assay as recited in claim 17 wherein the cell culture is negative for the selectable marker.

19. The assay as recited in claim 17 wherein the retrovirus is selected from the group consisting of human immunodeficiency virus type 1, human immunodeficiency virus type 2, feline immunodeficiency virus, simian immunodeficiency virus, visna-maedi virus, caprine arthritis-encephalitis virus, equine infectious anemia virus, and bovine immune deficiency virus, and hybrids thereof.

20. The assay as recited in claim 17 wherein the retrovirus is human immunodeficiency virus type 1.

21. The assay as recited in claim 17 wherein the selectable marker gene comprises a hygromycin B resistance gene and the medium selectable for the selectable marker comprises hygromycin B.

22. The assay as recited in claim 21 wherein the hygromycin B resistance gene comprises hygromycin B phosphotransferase gene.

23. The assay as recited in claim 17 wherein the mutation target gene is a thymidine kinase gene and the medium selectable for the mutation target gene comprises bromodeoxyuridine or HAT.

24. The assay as recited in claim 17 wherein the medium selectable for the mutation target gene comprises bromodeoxyuridine.

25. The assay as recited in claim 17 wherein the cell culture comprises 143B cells.

-32-

26. The assay as recited in claim 17 wherein the assay further comprises providing a negative control group, wherein a quantity of naïve cells from the target cell culture is left uninfected and placed under selection with a medium selectable for the selectable marker plus a medium selectable for the mutation target gene.